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Volatile fatty acids accumulation and rhamnolipid generation *in situ* from waste activated sludge fermentation stimulated by external rhamnolipid addition

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ABSTRACT

The solubilization and acidification of waste activated sludge (WAS) were apparently enhanced by external rhamnolipid (RL) addition. The maximum solute carbohydrate concentrations increased linearly from 48 ± 5 mg COD L^{-1} in the un-pretreated WAS (blank) to 566 ± 19 mg COD L^{-1}, and protein increased from 1050 ± 8 to 3493 ± 16 mg COD L^{-1} at RL dosage of 0.10 g g^{-1} TSS. The highest VFAs concentration peaked at 3840 mg COD L^{-1} at RL dosage of 0.04 g g^{-1} TSS, which was 4.24-fold higher than the blank test. RL was generated *in situ* during WAS fermentation when external RL was added. It was detected that RL concentration was increased from initial 880 ± 92 mg L^{-1} to 1312 ± 7 mg L^{-1} at the end of 96 h with RL dosage of 0.04 g g^{-1} TSS, which was increased to 1.49-fold. Meanwhile, methane production was notably reduced to a quite low level of 2.0 mL CH_4 g^{-1} VSS, showing effective inhibition of methanogens by RL (58.8 mL CH_4 g^{-1} VSS in the blank). In addition, the activity of hydrolytic enzymes (protease and α-glucosidase) was enhanced accordingly. VFAs accumulation and RL generation *in situ* demonstrated that the additional RL substantially performed enhanced biological effects for waste activated sludge fermentation.

1. Introduction

The problem of increasing energy demand and depleting fossil fuel will become more acute in the next decades [1]. It is necessary to research and develop alternative renewable energy sources before supplies become severely constrained. Nowadays, waste activated sludge (WAS) is increasingly attracted on utilization as a cheap but valuable energy resource which was treated sustainably with many environmental benefits [2]. As important high added-value chemical materials and the most preferred substrates for many bioprocesses, volatile fatty acids (VFAs) can be successfully produced from WAS during anaerobic digestion process [3]. Many researchers have conducted studies for the utilization of sludge fermentation liquid for biological nutrient removal [4], polyhydroxyalkanoates production [5], hydrogen production by MEC reactor [6] and so on.

Generally, anaerobic digestion of the sludge organics is considered to be a three-step process occurring sequentially: hydrolysis, acidification and methanogenesis. Hydrolysis is known as the rate-limiting step when degrading this type of complex organic material [7]. Previous researches had shown that only 30%–50% of the total chemical oxygen demand (TCOD) or volatile solids in WAS would be biodegraded within 30 days if the particulate organic matter was not properly disintegrated [8]. Various pretreatment methods for enhancing WAS solubilization were previously studied including chemical, mechanical, enzymatic and thermal treatments [9]. Nowadays, the addition of surfactants was regarded as an alternative strategy for WAS pretreatment due to its unique characteristic [10]. Surfactants are amphiphilic compounds with a hydrophilic and hydrophobic moiety, which can reduce the interfacial tension and form micelle aggregates. However, concerns over some chemically synthetic surfactants have increased, because most of them, such as Tween80, Triton X-100, sodium dodecyl sulphate (SDS) and sodium dodecyl benzene sulfonate (SDBS) would accumulate in the treatment system and thus present a potential risk for environment and human health [11]. Due to the specific properties of biodegradability, biocompatibility and low toxicities, biosurfactants were more desirable in environmental applications [12].

Biosurfactants can be biologically produced by many different microorganisms and are grouped as: glycolipids, phospholipids,

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Table 1
The main characteristics of the concentrated WAS.

Parameter	Value[a]
pH	6.42~6.62
C/N ratio	5.92 ± 0.19
TSS (total suspended solids)	21,840 ± 720
VSS (volatile suspended solids)	11,962 ± 372
SCOD (soluble chemical oxygen demand)	819 ± 62
TCOD	20,127 ± 341
Total VFAs (as COD)	212 ± 36
Total carbohydrate (as COD)	1409 ± 129
Total protein (as COD)	10,072 ± 396
Solute carbohydrate (as COD)	55 ± 7
Solute protein (as COD)	375 ± 14
lipid and oil (as COD)	273 ± 42

[a] All values are expressed in mg L^{-1} except pH and C/N radio.

polysaccharide–lipid complexes, lipoproteins–lipopeptides, hydroxylated and cross-linked fatty acids based on the producing microorganism [13]. Currently, many studies have been focused on utilizing biosurfactants on hydrocarbons biodegradation, tertiary petroleum recovery, metals removal from soil and so on [14]. As a kind of most known glycolipid biosurfactants, rhamnolipids (RL) are microbially produced by *Pseudomonas sp.* bacteria as anionic amphiphilic molecules, with (hydrophilic) mono- or di-rhamnose sugar heads and (lipophilic) ß-hydroxyalkanoic acid tails of varying lengths. To date, RL are of increasing industrial interest because of their great environmental compatibility, excellent surface activity, prominent foaming/wetting properties and broad range of potential applications [15].

Although it has been observed that sludge hydrolysis and acidification can be enhanced in the presence of RL, WAS subjected to RL pretreatment has not been comprehensively characterized. Also, the optimal RL dosage for maximizing VFAs production from WAS fermentation remain undetermined. Furthermore, the effect of RL on the methane production has not been investigated. The variations of RL with time during WAS fermentation was also need to be studied. Therefore, in this study, the accumulation of VFAs by fermentation of RL-pretreated WAS was evaluated in lab-scale reactors. The effect of RL dosage on VFAs composition and particulate organics solubilization was studied. In addition, the variation of RL with time was monitored in order to demonstrate RL decomposition in the WAS fermentation.

2. Materials and methods

2.1. Source of waste activated sludge

Raw WAS was collected immediately from the secondary sedimentation tank of Taiping Municipal Wastewater Treatment Plant (Harbin City, Heilongjiang Province, China). The sludge was concentrated by settling for 24 h, and stored at 4 °C in a refrigerator for less than 2 weeks. To prevent clogging problems, the sludge was screened with a 1 mm sieve to remove impurities prior to be used as feed. The main characteristics (average value plus standard deviation of three tests) of the concentrated WAS were displayed in Table 1.

2.2. Biosurfactant

Eighty percent RL solution (Victex Company, China) was used in the experiments, which was a blend of $RhaC_{10}C_{10}$ ($C_{26}H_{48}O_9$, m/z 503) and $RhaRhaC_{10}C_{10}$ ($C_{32}H_{58}O_{13}$, m/z 649). The critical micelle concentration (CMC) of this cell-free culture broth was detected to be 13.16 mg RL L^{-1} with a minimal surface tension of 30.45 mN m^{-1} by the ring method. The chemical structure of the rhamnolipid blend was shown in Fig. 1.

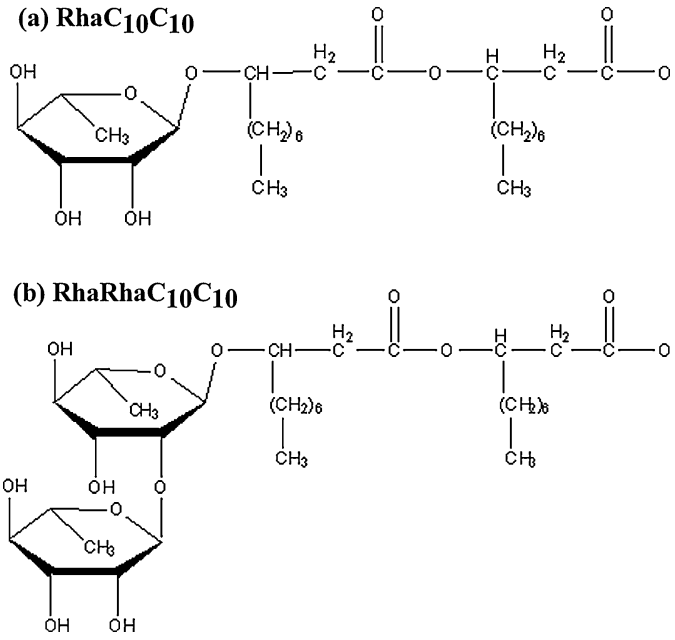

Figure 1. Chemical structures of rhamnolipid.

2.3. Batch fermentation set-up

Batch laboratory-scale anaerobic fermentation experiments were conducted in 500 mL serum bottles filled with 300 mL raw sludge each. The RL dosages ranging from 0.005 to 0.10 g RL g^{-1} TSS was applied to the sludge. The blank tests were conducted without RL simultaneously. Nitrogen gas was flushed to remove oxygen, and all bottles were capped, sealed, and stirred in an air-bath shaker (100 rpm) at 35 ± 1 °C for 8 d. All the fermentation experiments were carried out in triplicate.

To investigate the effect of RL addition on the methanogenesis step of anaerobic treatment for WAS, the following fermentation tests with synthetic wastewater containing sodium acetate (NaAc) were conducted. The RL dosage of 0.04 g g^{-1} Ac was applied, the control tests were conducted without RL simultaneously. The chemical composition of synthetic wastewater is: NaAc (1.0 g L^{-1}), potassium phosphate (50 mM, pH 7.0), KCl (0.13 g L^{-1}), NH_4Cl (0.31 g L^{-1}), Wolf's trace element solution (0.5 mL L^{-1}) and vitamin solution (0.5 mL L^{-1}). The Wolf's trace element solution and vitamin solution are described in [16]. Activated sludge of a UASB reactor from the Taiping WWTP in Harbin, China was used as the inoculum. All other operations were the same as described above.

2.4. Analytical methods

Sludge samples were centrifuged at a speed of 10,000 rpm min^{-1} after fermentation, then filtered through a 0.45 μm cellulose nitrate membrane filter and finally stored at 4 °C prior to analysis. The filtrate was immediately used to analyze VFAs, carbohydrate and protein. The determinations of SCOD, TCOD, carbohydrate, protein, TSS and VSS were the same as described in our previous publications [17]. The pH value was measured by a pH meter (Seven Multi, Mettler Toledo, Switzerland). A gas chromatography (GC) was utilized to analyze the composition of VFAs [6]. The VFAs production was calculated as the sum of the measured acetic (HAc), propionic (HPr), n-butyric (n-HBu), iso-butyric (i-HBu), n-valeric (n-HVa) and iso-valeric (i-HVa) acids.

Cali-5-Bond™ gas-sampling bags (1 L) were used to collect biogas produced. The total volume of gas was measured using a glass syringe. Gas composition was analyzed using another GC